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(54) LYMPHOCYTE-STIMULATION MATERIAL FOR REMEDY OF CANCER

(57)Abstract:

PURPOSE: To obtain the titled stimulation material by bonding a specific substance such as interleukin 1 to an insoluble carrier through covalent bond.

CONSTITUTION: The objective lymphocyte-stimulation material for the remedy of cancer can be produced by bonding (A) one or more substances selected from the following four substances (interleukin 1, OK432, interleukin 2 produced by genetic engineering method, and γ-interferon) to (B) an insoluble carrier (inorganic carrier such as activated carbon, glass, etc.; carrier originated from natural polymers such as cellulose, Sepharose, etc.; or synthetic polymer such as polystyrene, polyethylene, etc.) through covalent bond. Lymphocyte means hematocyte other than erythrocyte and platelet and includes the cell fraction obtained by removing granulocyte or B-cell from the lymphocyte.

EFFECT: The stimulation agent is effective to stimulate and activate lymphocyte and induce a strong antitumor immune cell in high safety and operability, and is useful for the remedy, inspection, diagnosis, research, etc., of gastric cancer, pulmonary cancer, mammary cancer, etc.

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LYMPHOCYTE-STIMULATION MATERIAL FOR CANCER TREATMENT
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Specifications

1. Title of the Invention

Lymphocyte-Stimulation Material for Cancer Treatment

2. Claim(s)

A lymphocyte-stimulation material for a cancer treatment characterized by bonding four substances—interleukin 1, OK432, interleukin 2 obtained by virtue of genetic engineering and γ-interferon to a one or more insoluble carriers by covalent bonding.

3. Detailed Specifications

(Field of Industrial Application)

The present invention relates to a lymphocyte-stimulation material for treating cancer having a function for inducing antitumor immunocytes by activating leucocytes.

(Prior Art)

As is well known, it has been reported that cells, such as killer T cells, NK cells, activated macrophages and K cells play an important role as antitumor immunocytes in immunological surveillance against malignant tumors in the living body (Masahiro Fukuzawa Igaku No Ayumi 126 (1983):420). Consequently, activating immunocytes (leucocytes) of patients with cancer to induce the above-mentioned antitumor immunocytes has been considered as an immunotherapy method for malignant tumors. However, although an immunological surveillance mechanism against malignant tumors actually exists in the living bodies of cancer patients, the tumor cells proliferate.

Activation for inducing immunosuppressor cells (suppressor T cells, suppressor macrophages, etc.) by tumor cells has been reported as a chief mechanism (S. Fujimoto, et al. J. Immunol. 116 (1976):791).

It is thought that such immunosuppressor cells suppress activity for inducing various antitumor immune cells that function in impairing antitumor cells, thus allowing the proliferation of tumor cells, and increasingly inviting an immuno competence against tumors as a result. In addition, the possibility that an immuno response against tumor cells is suppressed due to the production of immunosuppressive factors by tumor cells also has been reported (J.A. Roth, et al. J. Immunol. 128 (1982):1955) as another mechanism. It must be said that the activation for inducing effective antitumor immunocytes in the living bodies of cancer patients under a state of such immunosuppression is difficult.

As a consequence, a method for treating cancer by setting the optimum conditions for activating and inducing antitumor immunocytes without any immunosuppression outside the body, inducing powerful antitumor immunocytes and putting them back in the original patient is thought to have the possibility of being a new, highly effective cancer immunity treatment.

(Problems to be Solved by the Invention)

Although no research is being performed actively at present in an attempt to treat cancer by inducing and activating antitumor immunocytes by stimulating and activating leucocytes removed to outside the body, then administering this into a cancer-carrying organism, tumor cells extracted from a cancer-carrying organism have been used to stimulate and activate leucocytes, but the operation is extremely complicated.

(Means for Solving the Problems)

As a result of painstaking research to solve the aforesaid problems, the inventors of the present invention discovered an astonishing finding that powerful antitumor immunocytes could be induced by stimulating and activating leucocytes with a stimulation material which fixes interleukin 1, OK432, interleukin 2 obtained by virtue of genetic engineering, and γ-interferon to an insoluble carrier by covalent bonds, which led them to achieving the present invention.

That is, the present invention pertains to a lymphocyte-stimulation material for a cancer treatment characterized by bonding four substances—interleukin 1, OK432, interleukin 2 obtained by virtue of genetic engineering and γ-interferon to a one or more insoluble carriers by covalent bonding.

In the present invention the substances bonded to the insoluble carrier include interleukin 1, OK432, interleukin 2 obtained by virtue of genetic engineering and γ-interferon, but of these, interleukin 2 and OK432 preferably induce antitumor immunocytes, and further, it is preferable that interleukin 2 has powerful inducing ability. The interleukin 1 used in the present invention may be obtained from leucocyte cultivation, and moreover, it is not obtained by virtue of genetic engineering.

Any hydrophilic or hydrophobic carrier can be used for the insoluble carrier used in the present invention, but when a hydrophobic carrier is used, a nonspecific adsorption of the serum components develop on the carrier; hence, more preferable results are given with a hydrophilic carrier. Any known shape such as a particulate, fibrous, hollow filamentous, and

filmy, also can be used for the shape of the insoluble carrier.

In order to immobilize a ligand used for the material of the insoluble carrier, the carrier can be activated, and should be stabilized physically by way of all steps including the activation reaction and immobilization reaction of the carrier. Specifically, in inorganic-based carriers include activated charcoal, glass, and the like as well as their derivatives. Natural polymer-derived copolymers include simple polysaccharides, such as cellulose, Sepharose, dextran, a starch, alginic acid, and chitin, and their derivatives; complex polysaccharides, such as agar-agar, pectin, konyaku [jelly made from starch of devil's tongue or pressed vegetable] and gum Arabic, and their derivatives; proteins, such as wool and silk proteins, and their derivatives, but these are used as the carrier after an activating treatment, such as a crosslinking reaction, as needed.

In addition, polymers and copolymers of vinyl-based polymers, such as styrene, vinyl acetate, methacrylic acid ester, acrylic acid ester, vinyl halides, vinylidene halides, acrylonitriles, acrylamides, methyl vinyl ketone, vinyl pyrrolidone, 2-vinylpyridine, ethylene, propylene, butadiene, isoprene, and the like, and their derivatives may be exemplified for synthetic polymers.

Although a so-called known method, such as covalent bonding, ion bonding or physical adsorption, may be used for a method in which interleukin 1, OK432, interleukin 2 obtained by virtue of genetic engineering or γ-interferon is fixed to the surface of the insoluble carrier as a ligand, in consideration of the solubility, it is favorable to use a method of fixing by covalent bonding. Therefore, methods generally used for

immobilizing enzymes and in affinity chromatography may be employed. For example, a method in which an insoluble carrier is epoxy-activated and a ligand is bonded thereto, among other methods may be employed. In addition, as needed, a method in which molecules (spacers) of any given length can be introduced between the insoluble carrier and the ligand(s) can be employed.

The method for manufacturing the stimulation material is not limited to the above-mentioned methods. For example, methods, such as a method in which an oligosaccharide is bonded to a vinyl monomer and this is polymerized and a method in which a ligand is activated and bonded to the carrier, may be employed. Thus, the present invention is not limited to a method for manufacturing the stimulation material.

In the present invention, the term "leucocyte" is intended to mean the so-called blood's white cell which are blood cells excluding erythrocytes and thrombocytes and a cell fraction obtained by removing granulocytes and/or B cells from the so-called blood's white cells. The leucocytes activated in the present invention may use the leucocyte fraction gathered from peripheral blood in a known continuous centrifugation method, and moreover, they may be a mononuclear cell fraction separated in a known Ficoll-Paque centrifugation method, even if a T cell fraction separated and concentrated from peripheral blood mononuclear cells by a rossette formation with known neuramidase-treated sheep erythrocytes, powerful tumor-impairing cells can be induced.

The induced and activated tumor-impairing cells in the present invention belong to the lymphocyte fraction excepting granulocytes,

monocytes and macrophages, and above all, they have T cell properties.

If activation of the peripheral blood leucocytes by the stimulation material is performed on a serum component-containing medium or a medium having interleukin 2 added thereto, powerful tumor-impairing cells can be induced. That is, a medium containing 2 to 20% of an animal serum, such as fetal calf serum, bovine serum or equine serum, or human serum is prepared. Preferably, a medium in which 2 to 20% serum is contained is prepared. A medium used commonly for animal cell culture, such as RPMI 1640 medium, MEM medium, and so forth can be used for the medium in this case. Moreover, even an RPMI 1640 medium in which a serum component, such as serum albumin, has been added also can be used.

Peripheral blood leucocytes collected in various methods are suspended on the prepared medium at a cell concentration of 0.5 to 2×10^6 cells/mL, the proper amount of stimulation material is added thereto, and this is cultured at a temperature of 25 to 45°C. If this is performed at a temperature of less than 25°C, effective activation of the leucocytes does not occur at all. Culturing is simple as long as a commercially-available plastic container used for culturing cells is used in a CO_2 incubator. After culturing for 1 to several days, the activated leucocytes are recovered. Or, a long-term culturing may be performed on an interleukin 2-containing medium.

It was proven that the activated leucocytes thus obtained powerfully killed tumor cells.

(Advantages of the Invention)

The stimulation material of the present invention, as stated above, stimulates and activates leucocytes, it is safe, has good workability, induces powerful antitumor immunocytes, and can be used in treating gastric cancer, lung cancer, breast cancer, and the like, as well as in the detection and diagnosis, research, and the like thereof.

Practical Examples

100 μg respectively of interleukin 2 obtained by virtue of genetic engineering and γ-interferon (Recombinant IL-2, γ-IFN) or 1 mg OK432 (Picibanil; Chugai Pharmaceutical Co., Ltd.) was bonded, as a ligand, to 1 mL of a commercially-available CrBr-activated Sepharose (made by Pharmacia Fine Chemicals AB) in a known method to create a stimulation material. Moreover, in order to find the amount of this ligand thus retained, the amount of ligand in the supernatant and in the lavage was found after the bonding reaction. Upon calculating it by subtracting these from the amount of addition thereof, 95% of the ligands were retained.

Human leucocytes were obtained in the following manner. That is, drawn human peripheral blood is diluted to 1:2 with Hanks' solution, layered on a Ficoll-Paque solution (made by Pharmacia Fine Chemicals AB), centrifuged for 20 minutes at 2,000 rpm, the leucocyte layer in the intermediate layer is separated, this is washed with Hanks' solution, and suspended on a 10% autologous serum-containing RPMI 1640 medium (Nissui) at a concentration of 2×10^6/mL cells/mL. 1 mL of this cell suspension is fractionated into each of the 2 mL wells (Falcon No. 3047) used for cell culturing, and 50 μL of the stimulation material is added thereto and culturing is performed

in a CO_2 incubator at 37°C. Culturing is performed for 3 days, after which the mixture is pipetted and allowed to stand still to precipitate a carrier on the bottom of the container to thereby remove the supernatant serum, which was then washed with Hanks' solution, and subsequently suspended on a 10% autologous serum-added RPMI 1640 medium at a cell concentration of 5×10^6/mL cells/mL.

Whether or not these activated leucocytes had tumor cell-impairing properties was evaluated using a killer activity measurement method, as follows. Various human cancer cell strains adhered to a culture plate and propagated as target cells are suspended on a 10% fetal calf serum-added RPMI 1640 medium at a cell concentration of 5×10^4/mL cells/mL, 10 μL of this is fractionated into 10 μL Teraski plate, and cultured in a CO_2 incubator at 37°C for 24 hours. Upon completion of the incubation, the cancer cells stick strongly to the bottom of the culture plate. After this is washed with a culture liquid, 10 μL of a suspension of the activated leucocytes are added, cultured in a CO_2 incubator for 4 hours at 37°C, and the cancer cells adhering to the plate are kept from adhering. The cancer cells thus impaired lose their ability to adhere to the bottom of the plate, and removed along with the leucocytes when washed with Hanks' solution. The surviving cancer cells adhering to the bottom of the plate are fixed with acetone, stained with a Giemsa staining solution, and subsequently counted under a microscope. The killer activity is calculated according to the following expression.

Killer activity = {1-[(number of existing tumor cells when activated leucocytes added)/(number of surviving tumor cells when no activated leucocytes added)]}×100 (%)

Upon measuring the tumor cell-impairing ability of the immunocytes activated with each stimulation material, as shown in the table below, by employing such a method, a powerful impairing activity against MKN-1 human gastric cancer cells was exhibited.

Antitumor Immunocyte-Inducing Activity of Various Stimulation Materials

Stimulation Material	Impairing Activity Against HKN-1 Gastric Cancer Cells
IL-2-Sepharose	52%
γ-IFN-Sepharose	25%
OK432-Sepharose	50%

Comparative Example

Upon adding only a stimulation material-free or an insoluble carrier (Sepharose) and conducting the test as in the practical example, no antitumor immunocytes were induced at all.